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Branched-Chain Amino Acid Catabolism in Bacteria

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INTRODUCTION

The enzymatic conversions necessary for the catabolism of branched-chain amino acids have been reported to occur in a wide variety of bacteria. However, most studies have been done using several species of *Pseudomonas*. Pseudomonads are richly endowed with extraordinary nutritional versatility, which enables them to catabolize a diverse array of organic compounds (64), including the branched-chain amino acids. In a recent review of the regulation of catabolic pathways in *Pseudomonas* (44), Ornston discussed the history, methods of approach, and evolutionary forces affecting catabolic pathways. This review will summarize the bacterial catabolism of leucine, isoleucine, valine, and several compounds whose catabolic pathways converge with those of the branched-chain amino acids. Readers interested in mammalian catabolism of amino acid carbon skeletons are referred to a review by Rodwell (54). The data published in recent years on branched-chain amino acid catabolism leave little doubt as to the authenticity of the proposed metabolic scheme (Fig. 1).

The implication of defects in branched-chain amino acid catabolism as foci for inborn errors of metabolism has given both clinical relevance and research impetus to the elucidation of these pathways. The initial clinical observation of this phenomenon was made by pediatricians (43, 63), who correlated the uremic excretion of branched-chain metabolites and progressive familial infantile cerebral dysfunction. This original documentation of branched-chain amino acid-related genetic disorders has been expanded by clinical observation and biochemical experimentation to include the catabolic dysfunctions listed in Table 1. These syndromes range from fulminating to inapparent. Readers interested in more complete descriptions of these metabolic anomalies in man are referred to references 43 and 63. The intrinsic difficulties of studying catabolic pathways of low enzymatic activity in man and the paucity of clinical material have led to the utilization of prokaryotic metabolic models. A better understanding of these prokaryotic metabolic pathways may yield more therapeutic regimens, resulting in alleviation of the inborn error. An example of clinical application of knowledge obtained from this basic research is the current therapeutic administration of biotin to patients suffering from 3-methylcrotonylglycinuria. The function of biotin was first elucidated by using bacterial 3-methylcrotonyl coenzyme A (CoA) carboxylase in *Achromobacter* grown on isovalerate

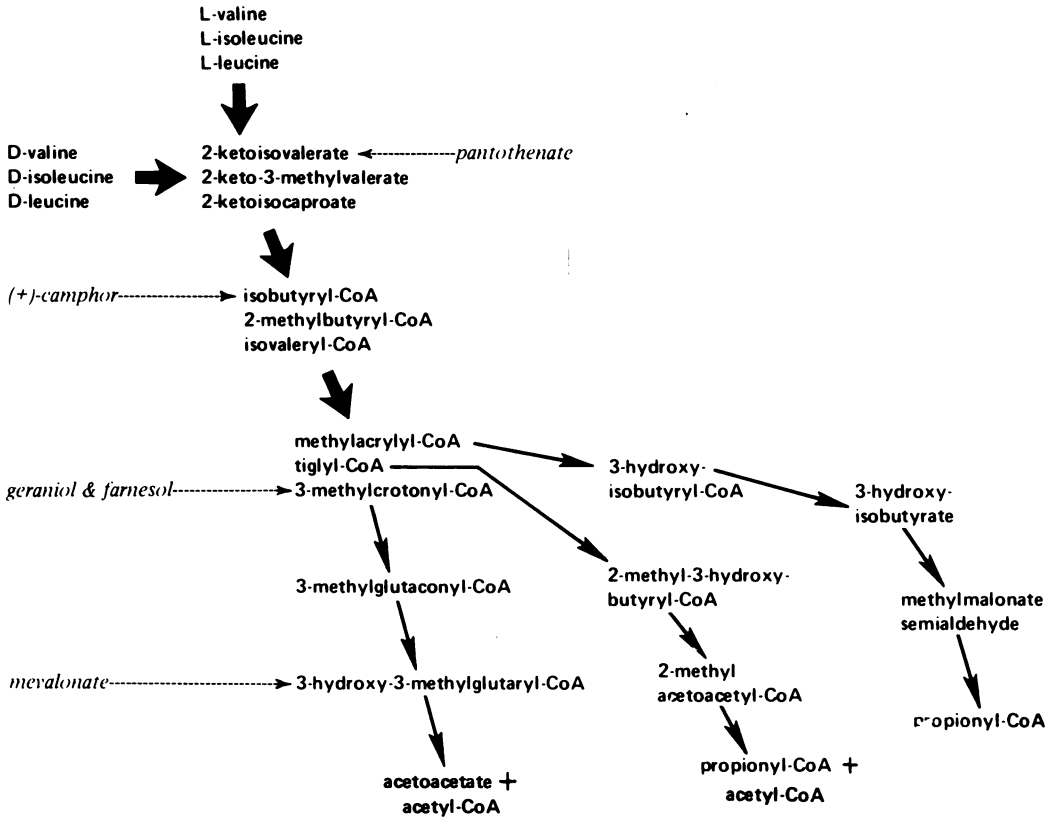

FIG. 1. *Pathway for the catabolism of D- and L-branched-chain amino acids in bacteria.*

(20). Some types of ketoacidemias may be similarly ameliorated by thiamine and vitamin B_{12}.

ENZYMES COMMON TO CATABOLISM OF ALL THREE BRANCHED-CHAIN AMINO ACIDS

The complete catabolism of each branched-chain amino acid requires the cooperation of two sequential series of reactions. The enzymes in the first series comprise a common pathway catalyzing the conversion of isoleucine, leucine, and valine to their respective acyl-CoA derivatives. The overlapping specificities inherent in this arrangement are advantageous to an organism. Metabolic redundancy is avoided since a single structural gene can control the catabolism of closely related analogues. However, branched-chain metabolites formed subsequent to this common pathway are catabolized by three separate enzyme series, one specific for each amino acid. Several other organic compounds, such as terpenes and long-chain alcohols, are degraded to metabolites identical to those found in amino acid-specific pathways. One advantage of separate enzyme series is that intermediates from converging pathways do not gratuitously induce nonessential enzymes of the common pathway.

TABLE 1. *Biochemical defects in genetic disorders of branched-chain amino acid catabolism*

Disorder	Biochemical defect
Maple syrup urine disease (5 variants)	Branched-chain keto acid dehydrogenase
Hypervalinemia	Valine transaminase
Isovaleric acidemia	Isovaleryl-CoA dehydrogenase
3-Methylcrotonylglycinuria	3-Methylcrotonyl-CoA carboxylase
Methylmalonic acidemia	
Type I	Methylmalonyl-CoA racemase
Type II	Methylmalonyl-CoA carbonylmutase
Type III	5-Deoxyadenosyl cobalamin synthesis
Type IV	Vitamin B_{12} formation (sulfur amino acid metabolism)
2-Methyl-3-hydroxybutyric aciduria	2-Methylacetoacetyl-CoA thiolase

This section will discuss the catabolism of isoleucine, leucine, and valine in a common pathway by branched-chain amino acid trans-

aminase, D-amino acid dehydrogenase, and branched-chain keto acid dehydrogenase. Although suggested by some data, there is insufficient evidence to make a categorical assignment of acyl-CoA dehydrogenase and enoyl-CoA hydratase to this common pathway. The physiological role of L-amino acid oxidase is even more obscure, although its end product would be metabolized via this common pathway.

Branched-Chain Amino Acid Transaminase

The ability to transfer an amino group between amino acid and keto acid is a metabolic necessity for both prokaryotic and eukaryotic organisms. Transamination is unique among enzymatic processes in that it is equally important in both biosynthesis and catabolism of amino acids.

Branched-chain amino acid transaminase (branched-chain amino acid 2-ketoglutarate aminotransferase; EC 2.6.1.42) was first described by Rudman and Meister (56), who separated two enzymes in *Escherichia coli* on the basis of their ability to transaminate different amino acids with 2-ketoglutarate. Transaminase A was most active with aromatic amino acids and oxalacetate, whereas transaminase B preferred branched-chain amino acids and 2-ketoglutarate acid.

Norton and Sokatch (37) purified branched-chain amino transaminase (transaminase B) from *Pseudomonas aeruginosa*. The enzyme was active with a number of amino acids over a wide pH range and appeared to be synthesized constitutively. Enzyme activity was limited to L-isomers. The Michaelis constants for amino and keto acid substrates were consistent with a possible dual function in both biosynthesis and catabolism. Voellmy and Leisinger (68) have recently reported such a dual-function transaminase. N^2-acetylornithine 5-aminotransferase from *P. aeruginosa* functions in both arginine catabolism and biosynthesis.

Martin et al. (33) isolated a *P. putida* mutant that was unable to catabolize valine, leucine, or isoleucine. However, the branched-chain keto acids supported growth. The mutant was not an amino acid auxotroph. A reduced level of branched-chain amino acid transaminase activity was the only discernible enzymatic difference between this pleiotropic mutant and the wild type. It was not determined whether the activity observed was due to a mutation in one of multiple transaminases with overlapping specificities or to alteration of a single bifunctional transaminase, with loss of only the catabolic activity. It is noteworthy that neither Martin et al. (33) nor Marinus and Loutit (29) were able to isolate *Pseudomonas* mutants completely devoid of branched-chain amino acid transaminase activity.

Puukka et al. (48) reported that branched-chain amino acid transaminase in *P. fluorescens* was inducible by combinations of branched-chain amino acids or their keto acids. They did not ascertain the mechanism for regulation of enzyme synthesis.

Coleman et al. (7, 8) purified branched-chain amino acid transaminases from *Salmonella typhimurium* and *S. montevideo*. No significant kinetic differences were found between the enzymes of the two *Salmonella* species. Detailed kinetic analysis indicated that transamination proceeded via a binary "ping-pong" mechanism. Transaminase mutants were isolated from *Salmonella* by Kiritani (23). He observed normal wild-type growth rates only when the minimal medium was supplemented with all three branched-chain amino acids. This phenomenon most likely reflects the biosynthetic role branched-chain transaminase plays in *Salmonella* metabolism.

L-Amino Acid Oxidase

Bernheim et al. (3) observed in 1935 that *Proteus vulgaris* cell suspensions oxidized most of the naturally occurring L-amino acids, including the branched-chain amino acids. Stumpf and Green (65) reported that the enzymes involved in the oxidation of 22 amino acids could be classified by differences of in vitro enzymatic stability. The most stable oxidase attacked only the L-isomers of the amino acids, producing the keto acid, ammonia, and water.

Two distinct particulate L-amino acid oxidases (L-amino acid:oxygen oxidoreductase [deaminating]; EC 1.4.3.2) were separated from *Proteus rettgeri* by Duerre and Chakrabarty (12). The substrate specificity of one enzyme was limited to basic amino acids and citrulline. The other oxidase had a broader specificity, catalyzing the oxidative deamination of monoaminomonocarboxylic, imino, aromatic, sulfur-containing, and 3-hydroxy,L-amino acids.

The in vivo role of L-amino acid oxidases in bacterial branched-chain amino acid catabolism is unknown. Stumpf and Green (65) detected L-amino acid oxidase in only three of ten genera they examined, indicating a limited distribution. Similarly, Coudert and Vandecasteele (11) surveyed several species and found L-amino acid oxidase activity in *Corynebacterium, Brevibacterium, Micrococcus,* and *Mycobacterium,* but not in *P. putida, E. coli,* or *Bacillus subtilis*. The oxidase from *Corynebac-*

terium was unusual since it was the only bacterial oxidase reported to produce hydrogen peroxide rather than water. Several L-amino acids induced the oxidase activity. These results imply that L-amino acid oxidase is neither ubiquitous nor essential to the metabolism of all bacteria.

D-Amino Acid Dehydrogenase

Another method of enzymatically producing branched-chain keto acids is via the oxidative deamination of D-stereoisomers of the amino acids. In bacteria, this reaction is catalyzed by D-amino acid dehydrogenase (EC 1.4.99.1). Prokaryotic D-amino acid deamination systems thus far reported differ mechanistically from their eukaryotic counterparts in that they utilize 0.5 mol of oxygen per mol of amino acid oxidized, implying that water rather than hydrogen peroxide is the final product.

Early studies of bacterial D-amino acid oxidation were made by Bernheim et al. (3) and by Stumpf and Green (Fed. Proc. 5:I157, 1946) with cell-free extracts from *Proteus*. Both groups reported utilization of 1 atom of oxygen per mol of amino acid oxidized. However, similar stoichiometry would result from the action of catalase on peroxide if both are present. Sokatch and his colleagues (31, 36) found that *P. aeruginosa* D-amino acid dehydrogenase preparations were free of catalase. Neither Yoneya and Adams (73) nor Stumpf and Green (65) were able to detect peroxide formation in their studies. By contrast, kidney D-amino acid oxidase (EC 1.4.3.3) consumes 1 mol of oxygen per mol of amino acid oxidized and forms peroxide as an end product.

Tsukada (66) partially purified two distinct D-amino acid dehydrogenases from *P. fluorescens*. One species was synthesized constitutively and had an absolute specificity for 2,6-dichloroindophenol as the electron acceptor. The other enzyme was induced by growth on D-tryptophan and was specific for methylene blue as the electron acceptor. Both dehydrogenases contained a flavin adenine mononucleotide (FAD) prosthetic group and were active with a number of D-isomers, including those of the branched-chain amino acids. Both enzymes were inactive with acidic amino acids. The two dehydrogenases were dissimilar in substrate specificity, electron acceptors, thermal lability, and inducibility, but had similar pH optima, absorption spectra, and enzyme kinetics. Neither enzyme was affected by the presence of divalent cations and chelating agents.

Norton and Sokatch (36) reported that cell-free extracts of *P. aeruginosa* grown on DL-valine catalyzed the oxidative deamination of D-valine to 2-ketoisovalerate. Enzyme activity was inducible and consumed 1 atom of oxygen per mol of keto acid produced. This line of investigation was continued by Marshall and Sokatch (31), who achieved a 13-fold purification of D-amino acid dehydrogenase. The partially purified enzyme preparation was active with the D-isomers of branched-chain, aromatic, and basic amino acids, but was inactive with acidic amino acids. Enzyme activity was exceedingly unstable and particulate in nature. The pH optimum varied as a function of the substrate. Comparative studies of the bleaching of D-amino acid dehydrogenase indicated the presence of cytochrome *c* and flavin. Biochemical experimentation and genetic analysis by Sokatch and his associates (32, 33) indicated that pseudomonal D-amino acid dehydrogenase is a regulatory segment separate from the other branched-chain amino acid catabolic enzymes.

Yoneya and Adams (73) described an inducible allohydroxy-D-proline oxidase from *Pseudomonas striata*. Strong similarities were noted between this enzyme and previously described D-amino acid dehydrogenases in absorption spectra, stoichiometry of oxygen consumption, particulate nature, broad substrate specificity, and induction by D-amino acids.

The presence of D-amino acid deamination enzymes raises questions about the role of the enzymes in bacterial metabolism. Presumably, functional enzymes exist because they are, or were, of survival benefit to the organisms. One might logically hypothesize that D-amino acid dehydrogenases evolved to aid the catabolism of the mucopeptide layer of bacterial cell walls. However, this premise is contraindicated by the observation that bacterial D-amino acid dehydrogenases characterized so far are conspicuously inactive with D-glutamate, a major component of the murein sacculus. Another paradoxical aspect of D-amino acid dehydrogenases is their characteristic broad substrate specificity, which includes certain amino acids, the D-isomers of which are not known to occur in nature. The physiological function of D-amino acid dehydrogenase remains speculative.

Branched-Chain Keto Acid Dehydrogenase

In bacteria, branched-chain keto acid dehydrogenase appears to be an enzyme complex that oxidatively decarboxylates all three branched-chain keto acids to their respective acyl-CoA derivatives. In contrast, Connelly et al. (4, 9) have reported that mammalian systems utilize two dehydrogenases: one specific for 2-ketoisovalerate (EC 1.2.4.4) and the other

acting on 2-keto-3-methylvalerate and 2-keto-isocaproate (EC 1.2.4.3).

Rudiger et al. (55) reported that partially purified branched-chain keto acid dehydrogenase in *Streptococcus faecalis* was a multi-enzyme complex similar to pyruvate and 2-keto-glutarate dehydrogenases. They demonstrated that the complex contained three different enzymes participating in the oxidation of branched-chain keto acids: decarboxylase, transacylase, and flavin lipoamide oxidoreductase. Sasaki (58) partially purified an enzyme from *Proteus vulgaris* that catalyzed the decarboxylation of branched-chain keto acids and required CoA and nicotinamide adenine dinucleotide (NAD) as cofactors. Partially purified branched-chain keto acid dehydrogenase complexes from *Bacillus subtilis* (35) and *S. faecalis* (55) oxidized all three branched-chain keto acids. Although all three branched-chain keto acids served as substrates, they were not equally active. Both bacterial enzymes preferred 2-ketoisovalerate and were least active with 2-ketoisocaproate. In contrast, cell-free extracts of *P. putida* most efficiently oxidized 2-keto-3-methylvalerate (30). The addition of 1 mM L-leucine, or L-isoleucine but not L-valine, caused a 3.5-fold increase in activity when 10 mM 2-ketoisovalerate was used as substrate.

Willecke and Pardee (71) isolated and described a *B. subtilis* mutant that was defective in branched-chain keto acid dehydrogenase. This mutant required a short branched-chain fatty acid derived from either leucine, isoleucine, or valine for growth. Their results suggested that in *B. subtilis* a single enzyme was responsible for the oxidative decarboxylation of all three branched-chain fatty acids. Namba et al. (35) found that the *B. subtilis* enzyme was constitutive, an observation consistent with its probable role in lipogenesis.

Martin et al. (33) characterized *P. putida* mutants that showed a simultaneous loss of ability to oxidize all three keto acids, indicating that one enzyme complex was involved in the oxidation of all three branched-chain amino acids.

In *P. putida,* the inducers of the dehydrogenase have been identified by Marshall and Sokatch (32) as the branched-chain keto acids rather than the amino acids. A mutant deficient in the production of keto acids from L-branched-chain amino acids also lacked dehydrogenase activity unless grown in the presence of at least one branched-chain keto acid. In *P. putida* the three keto acids were equally effective as inducers of branched-chain keto acid dehydrogenase.

Branched-Chain Acyl-CoA Dehydrogenase

Little is known about branched-chain acyl-CoA dehydrogenase in either prokaryotes or eukaryotes. Marshall and Sokatch (32) reported a constitutive, very low activity for isobutyryl-CoA dehydrogenase in extracts of *P. putida*. Only minor differences in activity were observed when either butyryl-CoA or isobutyryl-CoA was used as substrate. They did not determine whether the enzyme was specific for branched-chain acyl groups or was an expression of butyryl-CoA dehydrogenase (EC 1.3.99.2) activity in the beta oxidation of fatty acids. Engel and Massey (13) purified butyryl-CoA dehydrogenase from *Peptostreptococcus elsdenii* but only assayed with straight-chain acyl-CoA derivatives, so the enzyme's role in branched-chain catabolism is unknown.

ENZYMES SPECIFIC FOR INDIVIDUAL AMINO ACID PATHWAYS

Leucine

Three enzymes beyond the common pathway are required to complete the catabolism of leucine to acetoacetate. These leucine-specific enzymes are 3-methylcrotonyl-CoA carboxylase (EC 6.4.1.4), 3-methylglutaconyl-CoA hydratase (EC 4.2.1.18), and 3-hydroxy-3-methylglutaryl-CoA lyase (EC 4.1.3.4).

Lynen et al. (25) first demonstrated the carboxylation of 3-methylcrotonyl-CoA in extracts of *Mycobacterium* and *Achromobacter*. These bacteria were isolated from soil by using isovalerate as the sole source of carbon. Rilling and Coon (52) demonstrated the carboxylation of 3-methylcrotonyl-CoA in extracts of *Pseudomonas oleovorans*.

The biochemical function of biotin was elucidated by Himes et al. (20) through their studies of 3-methylcrotonyl-CoA carboxylase from *Achromobacter*. When the purified enzyme was incubated with adenosine 5'-triphosphate (ATP) and radioactive bicarbonate under appropriate conditions, a labeled carboxylated enzyme resulted. The enzyme complex then catalyzed the transfer of the carbonyl moiety to 3-methylcrotonyl-CoA, forming 3-methylglutaconyl-CoA. Massey et al. (34) demonstrated that 3-methylcrotonyl carboxylase in *P. putida* was inducible by growth on isovalerate as the sole carbon source. 3-Methylglutaconyl-CoA was enzymatically hydrated to 3-hydroxy-3-methylglutaryl-CoA. This hydration has also been reported in *Mycobacterium* (19) and *Achromobacter* (25).

The final enzyme specific for leucine catabolism, 3-hydroxy-3-methylglutaryl-CoA lyase, has been reported in an actinomycete grown on

mevalonic acid (61) and has been partially purified from *P. putida* (Massey and Sokatch, unpublished data).

Both 3-methylcrotonyl-CoA carboxylase and 3-hydroxy-3-methylglutaryl-CoA lyase were induced in *P. putida* grown on isovalerate. The addition of glucose or glutamate to cells growing on DL-leucine repressed the synthesis of both the carboxylase and lyase (34).

Winnacker and Barker (72) studied the metabolism of acetoacetate in *P. putida*. They demonstrated the presence of acetoacetyl-CoA:succinyl CoA transferase (EC 2.8.3.5) and acetoacetyl-CoA thiolase (EC 2.3.1.9) in crude extracts of cells grown on 3-amino-*n*-butyrate. The combined action of these enzymes completed the degradation of acetoacetate to acetyl-CoA and acetate. Their observations that isocitrate lyase and malate synthetase were induced during growth on 3-amino-*n*-butyrate led them to hypothesize that the resultant acetyl-CoA was metabolized via the glyoxylate cycle.

Pauli and Overath (45) reported a similar pathway of acetoacetate degradation in *E. coli*. The addition of acetoacetate to growth medium resulted in a 3,000-fold increase of acetoacetyl-CoA:succinyl-CoA transferase and acetoacetyl-CoA thiolase. The structural genes for these enzymes were closely linked to a regulatory gene.

Isoleucine

The bacterial oxidation of tiglyl-CoA to acetyl-CoA and propionyl-CoA was first described by Conrad et al. (10) in *P. putida*. Tiglyl-CoA hydratase and 2-methyl-3-hydroxybutyryl-CoA dehydrogenase were partially purified and characterized. The synthesis of these two enzymes and 2-methylacetoacetyl-CoA thiolase was induced by growth on either isoleucine, 2-keto-3-methylvalerate, 2-methylbutyrate, or tiglate. Inductive and kinetic analyses indicated that the catabolic hydratase and dehydrogenase were distinguishable from their beta-oxidative counterparts and are unique to the catabolism of isoleucine in *P. putida*. Enzyme activity was limited to 2-methyl-3-hydroxybutyryl-CoA, 3-hydroxybutyryl-CoA, and 2-hydroxy-3-methylvaleryl-CoA. The purified dehydrogenase had an absolute requirement for NAD and CoA esters. The enzymatic production of 2-methylacetoacetyl-CoA was authenticated by deacylation, chemical decarboxylation, and identification of methylethyl ketone hydrazone by thin-layer chromatography.

Valine

At least two enzymatic reactions, and perhaps as many as four, are known to be specific for the conversion of methylacrylyl-CoA to propionyl-CoA.

The first valine-specific enzymatic conversion is the hydration of methylacrylyl-CoA to 3-hydroxyisobutyryl-CoA. Puukka (46) reported that enoyl-CoA hydratase (EC 4.2.1.17) in *P. fluorescens* was induced by growth on branched-chain amino acids, branched-chain 2-keto acids, and short branched-chain fatty acids.

There is still no conclusive evidence that methylacrylyl-CoA hydratase (enoyl-CoA hydratase) is unique to the valine catabolic pathway. A hydration step is required for the catabolism of every branched-chain amino acid. Puukka (46) reported that isoleucine and leucine catabolic intermediates were more effective than valine as inducers of the hydratase when used as the sole source of carbon. The specificity of enoyl-CoA hydratases is discussed later in this review.

3-Hydroxyisobutyryl-CoA is enzymatically deacylated to free 3-hydroxyisobutyrate and CoA. Nurmikko et al. (41) and Marshall (30) have described this activity in *Pseudomonas*. Nurmikko et al. (41) reported that 3-hydroxyisobutyryl-CoA hydrolase (EC 3.1.2.4) was induced during the growth of *P. fluorescens* on either valine, 2-ketoisovalerate, isobutyrate, or 3-hydroxyisobutyrate. Isobutyrate and 3-hydroxyisobutyrate were the most effective inducers. Several Krebs cycle intermediates repressed the formation of the hydrolase. The uniqueness of 3-hydroxyisobutyryl-CoA hydrolase to valine catabolism has not been determined.

Puukka and Nurmikko (50) published studies on another valine-specific enzyme, 3-hydroxyisobutyrate dehydrogenase (3-hydroxyisobutyrate:NAD oxidoreductase; EC 1.1.1.31). The patterns of induction and repression for this enzyme and for 3-hydroxyisobutyryl-CoA hydrolase were similar. Bannerjee et al. (2) purified 3-hydroxyisobutyrate dehydrogenase 85-fold from *P. aeruginosa* grown on valine. The enzyme seemed to be specific to valine catabolism since, of a number of analogues tested, 3-hydroxyisobutyrate was the only substrate oxidized.

Methylmalonate semialdehyde is oxidatively decarboxylated to propionyl-CoA and carbon dioxide by methylmalonate semialdehyde dehydrogenase (EC 1.2.1.27), which was first characterized by Sokatch et al. (62) in extracts of *P. aeruginosa* grown on DL-valine. They purified the enzyme to a form homogenous by disc gel electrophoresis and analytical ultracentrifugation (2). The purified enzyme catalyzed the oxidation of either methylmalonate semialde-

hyde or propionaldehyde, concurrently with acylation of these substrates by CoA. The induction of methylmalonate semialdehyde dehydrogenase was studied in *P. fluorescens* by Puukka et al. (47) and in *P. putida* by Marshall and Sokatch (32). The enzyme was induced by either valine, 2-ketoisovalerate, isobutyrate, or 3-hydroxybutyrate. Kinetic analysis by Marshall and Sokatch (32) of 3-hydroxyisobutyrate dehydrogenase and methylmalonate semialdehyde dehydrogenase induction indicated that their synthesis was coordinately controlled.

SPECIFICITY OF ENOYL-CoA HYDRATASES

Although extensively studied in eukaryotic organisms, the physiological role of enoyl-CoA hydratases in prokaryotic catabolic pathways remains relatively unexplored. Our discussion will be limited to catabolism and excludes the enoyl-CoA hydratases of lipid biosynthesis, which have been discussed in other reviews (22). This may be an artificial division, since data clearly delineating the anabolic and catabolic hydratases are lacking. This qualification is particularly noteworthy since the carbon skeletons of all three branched-chain amino acids are incorporated in toto into branched long-chain fatty acids in a number of bacterial species, including *B. subtilis* (21) and *Micrococcus lysodeikticus* (M. G. Macfarlane, Biochem. J. 79:4P, 1961).

Conrad et al. (10), during a study of isoleucine catabolism in *P. putida*, partially purified and characterized a hydratase catalyzing the hydration of tiglyl-CoA (2-methylcrotonyl-CoA) and crotonyl-CoA. The corresponding enoyl-CoA derivative from leucine catabolism, 3-methylglutaconyl-CoA, was not hydrated, suggesting that *P. putida* requires at least two hydratases to catabolize branched-chain amino acids. Increased tiglyl-CoA hydratase activity was found in cells grown in the presence of isoleucine or isoleucine catabolic intermediates. Valine and leucine were slightly inductive. Conrad also noted that extracts of crotonate-grown cells had increased levels of crotonyl-CoA hydratase without a corresponding increase in the level of tiglyl-CoA hydratase. These data implied that the hydration of crotonyl-CoA and tiglyl-CoA is catalyzed by separate inducible hydratases possessing mixed substrate specificities. Thus *P. putida* crotonase activity was limited to crotonyl-CoA, but tiglyl-CoA hydratase was active with both crotonyl-CoA and tiglyl-CoA.

Puukka's (46) investigations of valine catabolism by *P. fluorescens* documented induction of methylacrylyl-CoA hydratase by growth on branched-chain amino acids, branched-chain 2-keto acids, and short branched-chain fatty acids. Highest levels of enoyl-CoA hydratase were detected when the carbon source was isoleucine or 2-ketoisocaproate. The specificity of the enzyme remains uncertain since methylacrylyl-CoA was the only substrate tested.

Waterson et al. (69) purified crotonase from *Clostridium acetobutylicum* without ascertaining its function, if any, in branched-chain amino acid catabolism. Their studies indicated that crotonase was one of multiple enoyl-CoA hydratases found in crude extracts. Clostridial crotonase was specific for crotonyl-CoA and hexenoyl-CoA. Although some molecular and catalytic homologies with bovine crotonase suggest a distant phylogenic relationship, evolutionary pressures have resulted in enzymes with distinct substrate specificities.

Weeks et al. (70) described an enoyl-CoA hydratase in *E. coli* that was active with crotonyl-CoA. The hydratase was coordinately induced with beta oxidation enzymes when the cells were grown on long-chain fatty acids. Crotonase induction was not observed in amino acid medium, which suggests that its activity in *E. coli* is limited to beta oxidation of fatty acids.

Seubert and Fass (59, 60) enriched isohexenylglutaconyl-CoA hydratase (EC 4.2.1.57) 100-fold from *Pseudomonas citronellolis*. They found that this enzyme functioned in the catabolism of long branched-chain alcohols. The enzyme was active with unsaturated dicarboxylic acid derivatives of geraniol and farnesol.

The available data suggest that the enoyl-CoA hydratases functioning in catabolism of branched-chain acids are probably distinct enzymes from the anabolic hydratases active in lipid biosynthesis. The *Pseudomonas* catabolic enoyl-CoA hydratases thus far reported have all been inducible.

CATABOLIC PATHWAYS CONVERGING WITH BRANCHED-CHAIN AMINO ACID PATHWAYS

Geraniol and Farnesol

Seubert and Fass (59, 60) described an interesting variation of the oxidation of branched-chain compounds. Geraniol and farnesol are long branched-chain alcohols that can be degraded by one species of *Pseudomonas* (Fig. 2). The catabolic pathway of these alcohols resembles beta oxidation, with additional carboxylation and cleavage steps similar to leucine catabolism. After conversion of geraniol and farnesol to the acyl-CoA derivatives, the methyl side chain of the resultant enoyl-CoA was carbox-

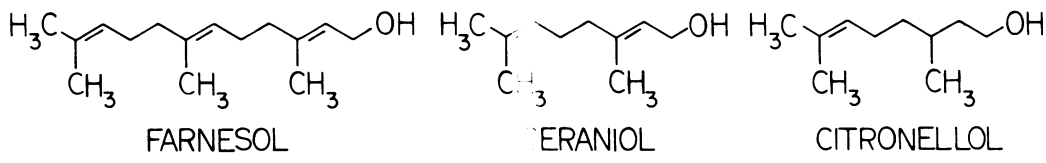

FIG. 2. *Long branched-chain alcohols metabolized by Pseudomonas citronellis.*

ylated. This was followed by hydration of the double bond. Seubert and Fass found that the purified enoyl-CoA hydratase was active with 3-methylcrotonyl-CoA as well as long-chain derivatives of geranyl-CoA and farnesyl-CoA. The cleavage of the hydroxylated CoA derivatives to acetate and a 3-ketoacyl-CoA derivative was catalyzed by hydroxyisohexenylglutaryl-CoA:acetate lyase (EC 4.1.3.26). The resultant 3-ketoacyl-CoA was metabolized by a pathway analogous to beta oxidation. Cyclic repetition of the carboxylation, hydration, and cleavage reactions eventually resulted in the production of 3-methylcrotonyl-CoA. The purified lyase described by Seubert and Fass only cleaved the hydroxy derivatives formed from geranyl-CoA and farnesyl-CoA. A separate lyase, hydroxymethylglutaryl-CoA:acetyl-CoA lyase (EC 4.1.3.4), is responsible for the cleavage of 3-hydroxy-3-methylglutaryl-CoA to acetyl-CoA and acetoacetate. The catabolic pathways for geraniol and farnesol were induced by citronellol, another branched-chain alcohol (Fig. 2).

Pantothenate

Goodhue and Snell (16) studied the bacterial metabolism of pantothenate by *Pseudomonas* P-2. They found that the incomplete oxidation of pantothenate resulted in the production of detectable amounts of beta-alanine, pantoate, valine, and 2-ketoisovalerate in culture filtrates. They explained the presence of these products by the hydrolysis of pantothenate to beta-alanine and pantoate, which was subsequently converted to 2-ketoisovalerate. They substantiated this pathway (Fig. 3) by partially purifying and characterizing the four necessary enzymes: pantothenate aminohydrolase, EC 3.5.1.22 (42); D-pantoate NAD oxidoreductase, EC 1.1.1.106 (17); D-aldopantoate dehydrogenase, EC 1.2.1.33 (26); and dimethylmalate: NAD oxidoreductase (decarboxylating), EC 1.1.1.84 (26).

Mäntsälä, Nurmikko, and others studied the regulation of pantothenate catabolism, including each of the reactions shown in Fig. 3, in *P. fluorescens* P-2 (27, 28, 38–40). They concluded that these four enzymes of pantothenate catabolism and pantothenate permease were coordinately induced by pantoate. All of the pantothenate catabolic enzymes were subject to

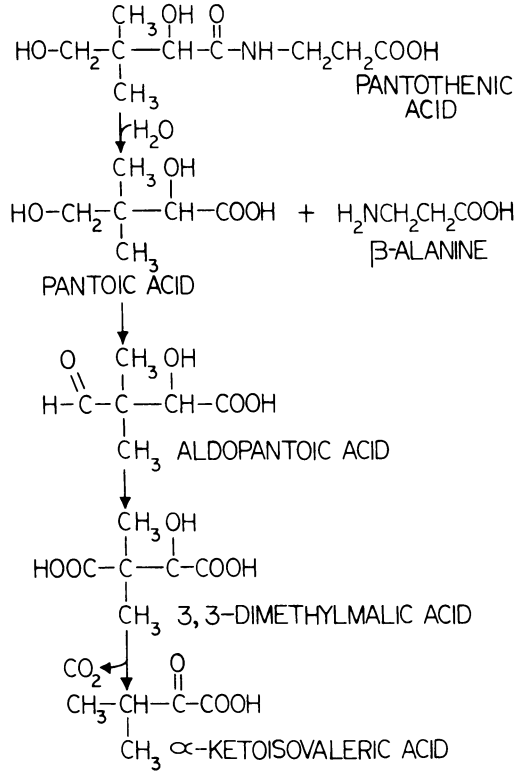

FIG. 3. *Pathway for the catabolism of pantothenic acid in Pseudomonas.*

repression by 2-keto acids and Krebs cycle intermediates.

The 2-ketoisovalerate formed from pantothenate was further metabolized in *P. fluorescens* P-2 by enzymes common to the catabolism of valine.

Mevalonate and 3-Hydroxy-3-Methylglutarate

Siddiqi and Rodwell (61) described the metabolism of mevalonate to 3-hydroxy-3-methylglutaryl-CoA in an actinomycete. Mevalonate was acylated and then oxidized to 3-hydroxy-3-methylglutaryl-CoA by a soluble NAD-requiring enzyme. Manometric experiments determined that at least one enzyme of mevalonate metabolism was induced by growth on mevalonate. Fimognari and Rodwell (14) partially purified mevalonate:NAD oxidoreductase (CoA acylating) (EC 1.1.1.34) from *Pseudomonas* M1

and *Mycobacterium*. Their kinetic analysis indicated that substrate binding to the enzyme required the presence of a carboxyl, a 3-methyl, and a 3-hydroxy group. Compounds with this configuration acted either as substrate or competitive inhibitor.

Ahmad and Siddiqi (1) isolated a pseudomonad capable of growing on 3-hydroxy-3-methylglutarate as the sole carbon source. Extracts of cells grown on 3-hydroxy-3-methylglutarate were acylated in the presence of ATP, CoA, and Mg^{2+}. The cell extracts did not transacylate 3-hydroxy-3-methylglutarate with succinyl-CoA. Cells grown on 3-hydroxy-3-methylglutarate contained 3-hydroxy-3-methylglutaryl-CoA lyase and oxidized acetoacetate, suggesting that the CoA derivative was catabolized by the appropriate leucine-specific enzymes.

Camphor

Camphor, a terpene, can be oxidized by some strains of *P. putida* to isobutyrate. Rheinwald et al. (51) demonstrated that the initial oxidative cleavage of camphor was catalyzed by enzymes coded on plasmid-borne genes, whereas isobutyrate catabolic enzymes were coded on chromosomal genes. Therefore, the complete oxidation of camphor by pseudomonads requires the participation of both plasmid-borne and chromosomal genetic segments. The camphor plasmid was readily accepted by most pseudomonads capable of using isobutyrate as sole carbon source. The isobutyrate portion of camphor was then catabolized by the valine-specific enzymes.

INHIBITION OF GROWTH BY BRANCHED-CHAIN AMINO ACIDS

The growth-inhibitory effects of amino acids incorporated into the nutritional media of heterotrophs were first noted in Gladstone's studies of *Bacillus anthracis* (15). Since that time (1939) numerous other analogous inhibitions have been documented. The first insight into this paradox of inhibition by essential nutrients was provided by Umbarger's observation (67) that the first enzyme unique to isoleucine biosynthesis was inhibited by the end product. The now familiar phenomenon of end product inhibition has been shown to be a ubiquitous regulatory mechanism in most biosynthetic pathways. Umbarger's extensive investigations of the molecular basis of branched-chain amino acid inhibitions in enteric bacteria have been responsible for much of the current understanding of these regulatory relationships. However, the regulation of branched-chain amino acid biosynthesis has been adequately reviewed elsewhere (67), and our review will be confined to inhibitions of growth observed when branched-chain amino acids are used as a sole source of carbon. This stipulation limits the discussion primarily to the pseudomonads, whose nutritional versatility enables them to catabolize a diverse array of natural and synthetic organic compounds. However, this metabolic versatility presents the cell with a regulatory dilemma when the sole source of carbon and energy can also function as a regulatory effector, essential nutrient, or metabolite in other pathways. The metabolism of branched-chain amino acids is a good case in point, since the branched-chain keto acids are both the first catabolic and last anabolic intermediates. These dual functions of keto acids raise the possibility of competition between anabolic and catabolic pathways. The diverging branched-chain amino acid biosynthetic and catabolic pathways are the epitome of cellular economy through utilization of common enzymes with overlapping specificities and shared metabolites. Generally speaking, this metabolic arrangement in a biological system is highly desirable since it eliminates unnecessary duplication of protein biosynthesis. However, this divergence may work to the detriment of the cell when excess end products, such as branched-chain amino acids, negate the synthesis of essential nutrients by repression, feedback inhibition, shunting of intermediates into catabolic pathways, or a combination thereof.

Inhibition of growth of *P. putida* by leucine has been previously reported (33, 34). There was a fourfold difference in growth rates obtained on the L- and D-isomers (20-h generation time versus 5 h). The metabolic basis for this disparity has not been elucidated, but possibly has its roots in a nutritionally induced upset of delicately balanced ratios of intermediates such as keto acids. The keto acid of leucine, 2-ketoisocaproate, is produced 50 to 100 times faster from the L-isomer by aminotransferase than from the D-isomer by D-amino acid dehydrogenase. This potential imbalance in branched-chain keto acid pools assumes relevance when the substrate specificity of 3-isopropylmalate synthetase, the first enzyme unique to leucine biosynthesis, is taken into account. Conrad and Jensen (Abstr. Annu. Meet. Am. Soc. Microbiol. P169, p. 172, 1974) found that the synthetase from *P. putida*, like that from *Salmonella* (24) and *Hydrogenomonas* (18), catalyzed the condensation of 2-ketoisocaproate and acetyl-CoA, synthesizing a possible antimetabolite. Inhibition of growth by L-leucine was reduced by either valine or its keto acid, 2-ketoisovalerate. The latter is the natural substrate for 2-isopropylmalate synthetase. 2-Isopropylmalate

synthetase from *P. putida* did not recognize the keto acid from isoleucine, 2-keto-3-methylvalerate. This is consistent with the observation that neither isoleucine nor its keto acid derivative relieved growth inhibition by L-leucine.

Extensive genetic mapping of *Pseudomonas* has delineated significant regulatory differences between the pseudomonads and enteric bacteria. The structural genes for the isoleucine-valine biosynthetic pathway in *P. aeruginosa* are scattered around the chromosome, in contrast to their juxtaposition in an operon in *E. coli* (29). These genetic differences do not rule out the possibility that the molecular bases for inhibition of growth are similar in the pseudomonads and enterics. However, these variances in growth may be manifestations of differences in catabolic capability.

Recent work by Calhoun and Hatfield (5) may necessitate other interpretations of the exact mechanisms of branched-chain amino acid inhibition. They proposed that the biosynthetic operon in *Salmonella* could be either repressed or induced by threonine deaminase (EC 4.2.1.16), depending upon the binding of threonine deaminase to either aminoacyl-transfer ribonucleic acid (tRNA) or biosynthetic intermediates. In addition, in vitro enzyme maturation could be modulated by isoleucine and valine. If this mechanism of autoregulation also functions in *Pseudomonas*, a preponderance of any one effector could result in a slower growth rate.

REGULATION OF DIVERGENT CATABOLIC PATHWAYS

Ideally, catabolic enzymes would be synthesized only in order to satisfy biosynthetic or energy demands. The degradation of pantothenate to propionate via 2-ketoisovalerate in *P. fluorescens* is an example of a catabolic pathway in which all the enzymes are inducible. An involved metabolic process such as this may be induced in segments, in order to avoid synthesis of early enzymes when an intermediate is available from other sources. *P. fluorescens* exhibits sequential induction by pantoate, 2-ketoisovalerate, isobutyrate, and 2-hydroxyisobutyrate. This form of metabolic regulation is apropos to the catabolism of rarely encountered nutrients and is consistent with the conservation of energy and essential metabolites.

Marshall and Sokatch (32) reported a different regulatory pattern for valine catabolism in *P. putida*. Branched-chain keto acid dehydrogenase, 3-hydroxyisobutyrate dehydrogenase, and methylmalonate semialdehyde dehydrogenase were induced by growth on valine. However, branched-chain amino acid transaminase and branched-chain acyl-CoA dehydrogenase appeared to be synthesized constitutively. The constitutive nature of a branched-chain amino acid transaminase may be appropriate if the enzyme is essential for both catabolism and biosynthesis in *P. putida*. Branched-chain acyl-CoA dehydrogenase has not yet been studied sufficiently to permit conclusions concerning its regulation.

In this review, we have presented data that substantiate the branched-chain amino acid catabolic pathway shown in Fig. 1. This type of branched pathway is unusual in bacterial physiology, since it consists of segments of enzymes common to the catabolism of leucine, isoleucine, and valine followed by three segments with enzymes specific for each amino acid. The oxidation of monophenols in *P. putida* is also accomplished by divergent catabolic pathways. Sala-Trepat et al. (57) described two dehydrogenases from *P. putida* that metabolized monophenolic intermediates by two mechanisms, but produced the same end product. This multiplicity of responses no doubt contributes to the immense catabolic capabilities of *Pseudomonas*.

There are several reasons for the recently revived interest in *Pseudomonas* metabolism. The recognition of the opportunistic pathogenicity and general antibiotic insensitivity of several species has given impetus to the study of their physiology. Comparative genetic and regulatory analyses between pseudomonads and enterics have indicated fundamental differences (6). Workers in the field expect that elucidation of these differences may give us an insight into basic regulatory mechanisms. The unsurpassed metabolic versatility and amenable genetics of *Pseudomonas* have made it the metabolic model in numerous investigations of catabolism. The study of human anomalies of branched-chain amino acid catabolism by bacterial prototypes has been conducted almost exclusively with pseudomonads. This review has summarized our present state of knowledge of bacterial catabolism of branched-chain amino acids. The knowledge and insight gained from these studies not only have added to a better basic understanding of metabolism, but also may be instrumental in formulating therapeutic relief of inborn errors of metabolism and alleviating pseudomonal disease.

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